Tailoring the Bioactivity of Titanium by Plasma Sprayed Hydroxyapatite-Bioactive Glass Composite Coating

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

Master of Technology

In

Biotechnology & Medical Engineering

By

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May 2013

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Certificate

This is to certify that the thesis entitled "**Tailoring the Bioactivity of Titanium by Plasma Sprayed Hydroxyapatite-Bioactive Glass Composite Coating**" by **Sandeep Sureshbhai Patel (211BM2225),** in partial fulfillment of the requirements for the award of the degree of Master of Technology in Biotechnology during the session 2011-2013 in the Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, is an authentic work carried out by him under our supervision and guidance. To the best of our knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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Acknowledgement

Successful completion of this project is the outcome of consistent guidance and assistance from many people, faculty and friends and I am extremely fortunate to have got these all along the completion of the project.

I owe my profound gratitude and respect to my project guide, Prof. A. Thirugnanam, Department of Biotechnology and Medical Engineering, NIT Rourkela for his invaluable academic support and professional guidance, regular encouragement and motivation at various stages of this project.

I also thank Mr. Mitun Das, Scientist C [Gr.IV(2)], Bioceramics & Coating Division, Central Glass & Ceramic Research Institute, Kolkata and Dr. Vamsi Krishna Balla, Senior Principal Scientist and Head of Bioceramics & Coating Division, Central Glass & Ceramic Research Institute, Kolkata. I am extremely grateful to them for their consistent guidance and support.

I place on record my sincere gratitude to Prof. Krishna Pramanik, Head of Department, Department of Biotechnology and Medical Engineering, NIT Rourkela for her constant encouragement.

I would like to thank Ms. Tejinder Kaur, Ph.D Scholar Department of Biotechnology and Medical Engineering, NIT Rourkela, for her regular support, help and motivation.

I would also thank my Institution and my faculty members without whom this project would have been a distant reality. I also extend my thanks to my family, friends, and well-wishers.

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Contents

Abbreviations

Ti……. ……Titanium

Cp Ti……Commercially pure titanium

HAp……..Hydroxyapatite

BG……Bioglass

SEM…….Scanning electron microscopy

XRD…….X-ray powder diffraction

MTT…… Methylthiazolydiphenyl-tetrazolium bromide

SBF……..Simulated body fluid

STA……..Solution treated and aged

PLD……..Pulsed laser deposition

LENS……Laser Engineered Net Shaping

ELI……Extra Low level of interstitial content

TCP……..β-tricalcium phosphate

c-SBF……...Corrected SBF

r-SBF……Revised SBF

n-SBF……...Improved SBF

TF-XRD……...Thin film X-ray diffraction

JCPDS…….. Joint Committee on Powder Diffraction Standards

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Abstract

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Keywords:

Titanium, hydroxyapatite, bioglass, plasma spray coating, bioactivity

Titanium and its alloys are widely used as a potential implant material due to their excellent mechanical and corrosion resistant properties. However, the bioinertness of titanium results in poor osseointegration of the implant which may result in failure. To improve osseointegration on titanium, several surface modification techniques using plasma spraying, chemical vapour deposition, etc have been carried out. Hydroxyapatite and bioglass coating has been proved to be one of the best coatings to improve surface bioactivity. Bonding strength of hydroxyapatite is more as compared to bioglass while bioactivity of bioglass is higher than hydroxyapatite. To improve the implant stability and bioactivity, composite of hydroxyapatite and bioglass containing SiO_2–Na_2O–CaO–P_2O_5 system was used as coating material. Micro plasma spray technique was used for coating different composition of the composite on commercially pure titanium substrate. The processing parameters were maintained at 2kW power, 7.3 g/min of feed rate and the current parameter was varied at 35A and 40A respectively. The coated specimens were characterized using optical microscopy, scanning electron microscopy (SEM) and X-ray diffraction (XRD) techniques. It was observed that there was no phase contamination on the coated specimens. The SEM micrographs revealed that the coated specimens exhibited microporoes on its surface. The *in-vitro* bioactivity study was performed by immersing the specimen in simulated body fluid (SBF) for 7 and 14 days. The immersed specimens were characterized using SEM and XRD to confirm apatite formation. It was evident that the results showed dense apatite formation on the coated specimens indicating enhanced bioactivity. From this study it is evident that composite (HAp+BG) coating showed more bioactivity than hydroxyapatite coating.

Chapter 1
Introduction

Chapter 1 Introduction

German chemist Martin Heinrich Klaproth, discovered Titanium metal confined in an ore in 1795. It was named "Titanium" after the titans of Greek mythology,as a symbol of power and strength. After aluminum, iron and magnesium, titanium is the fourth most abundant metal in the earth crust. Titanium has low density and high strength, good corrosion resistance to different media, good oxidation resistance and moderate strength at high temperature making it suitable for industrial applications. By changing the alloying and processing parameters the yield strength, fracture toughness and creep property of titanium can be increased. Titanium has allotropic phase transformation from high temperature β phase with body centered cubic structure to the room temperature α phase having closely packed hexagonal crystal structure. This transition temperature depends on alloy composition. Various elements forming solid solution with titanium are classified on the basis of their effect on the solubility of α and β phases. Elements like Al, Ga, O, N, C are α stabilizing element as they stabilize α phase while V, Mo, Nb, Fe, Cr, Ni etc. are β stabilizing elements as they stabilize β phase. Element like Sn and Zr are neutral, as they enter into solid solution with titanium without stabilizing α or β phase [1]. The only α stabilizer used for commercial purpose is aluminum.

Based on the alloying conditions and the phase present in the microstructure, titanium alloys are classified as a) alpha (α) alloys, b) near α alloys, c) α+β alloys, d) metastable β alloys, e) β alloys and f) titanium aluminides.

a) Alpha (α) alloys: Alpha titanium alloys are single phase alloys. Alpha stabilizers like aluminum and oxygen stabilize the α phase. Tin and zirconium are neutral elements and have solid solubility in both α and β phases. They also strengthen the α phase along with aluminum and oxygen. Alpha alloys have high stability and good high temperature properties, but cannot be heat treated for modification of microstructure for improving their properties. Commercially pure (CP) titanium and Ti-5Al-2.5Sn are the most important alloys of this type. Alpha alloys are mainly used in the chemical and process engineering industries due to their excellent corrosion resistance.

b) Near α alloys: Near α alloys mainly contain α stabilizers with some amount of β stabilizer in the final microstructure. Generally it contains around 1-2 wt% of β stabilizers which are added to

improve their strength and workability. The α phase is predominant in these alloys. These alloys find wide application in aero engines.

c) α+β alloys: These alloys contain large amount of β stabilizers around 4 to 6 wt%. Ti-6Al-4V and Ti-6Al-6V-2Sn are examples of this class of alloys. These alloys can be heat treated to develop a variety of microstructures and mechanical properties. Ti-6Al-4V, the commonly used alloy of this class is primarily used in α+β condition. These alloy are used in annealed or in solution treated and aged (STA) conditions. Ti-6Al-4V is a very well tested alloy and has a good balance of properties and hence widely used in the aerospace industries and biomedical applications.

d) Metastable β alloys: It contains 10 to 15 wt% of β stabilizers. The β phase is retained at room temperature in a metastable condition. These alloys have high strength, toughness, excellent hardenability and forgeabilty over a wide range of temperature. The α stabilizing elements are present in small amount as strengthening agents.

e) β alloys: In beta (β) alloys, the β phase is stabilized at room temperature by addition of approximately 30 % of β stabilizers such as V, Mo, Nb, Ta etc. These alloys have an excellent combination of fracture toughness and strength, which can be tailored by selecting appropriate aging temperature and time. Ti-10V-2Fe-3Al, Ti-13V-11Cr-3Al and Ti-15Mo are some of the prominent alloys of this type. β and near β alloys offer increased fracture toughness over α+β alloys at a given strength level.

f) Titanium aluminides: These alloys are based on the intermetallic compounds Ti_3Al (α_2) and TiAl (ϒ). The alloys based on Ti_3Al usually contains a large amount of Nb addition and other β stabilizers. These alloys possess excellent high temperature properties and good oxidation resistance, but have poor ductility and fracture toughness at room temperature.

Titanium and its alloys possesses good mechanical properties and corrosion resistance which allows it to find wide applications in aerospace industries, chemical industries as well as in biomedical implants. Because of high strength to weight ratio, easy formability, low modulus of elasticity and fatigue resistance, titanium have become an important material for aerospace industry since 1950[2], while its outstanding corrosion resistance property in corrosive environment is the prime reason for its use in chemical industries. Similarly owing to various characteristic properties associated with titanium and its alloys, different types of dental and

medical prostheses have been developed and are currently being utilized. They include cardiac mechanical heart valves and pacemaker, operational devices and equipment and orthopedic implants. Further in the dental field, the list can expand to orthodontic brackets and wires, dental implants, prosthetic appliances and endodontic files.

Commercially pure titanium (Cp Ti) and titanium alloy especially Ti6Al4V are widely used in the manufacturing of dental and orthopedic implants. Cp Ti and its alloys possess high oxidation number and high acidity that help in better osseointegration of metal with surrounding tissue environment. Apart from this they have excellent mechanical properties and corrosion resistance properties which plays important role in the subsequent biological process [3]. The bioactivity of titanium surface is not high enough to induce the direct growth of bone tissue, good bone fixation before several months [3, 4]. Also it is well recognized that the first reaction of vital hard/soft tissue i.e. host tissue to any type of biomaterial like ceramic, polymers, metals and composite is rejection, as biomaterial is normally recognized as a "foreign material" by the host tissue. The biological acceptances of these materials by the living tissues are essentially controlled by the surface and interfacial reaction between the organic substance and inorganic substrate. To maintain a controlled tissue interface importance of the implant surface with respect to tissue reaction has been recognized [5]. For shortening the time of bone fixation and controlling of tissue-titanium interaction several efforts were directed to the modification of metal surfaces [4].

Surface modification has been applied to metallic biomaterials in order to improve mechanical, chemical and physical properties such as wear resistance, corrosion resistance, biocompatibility and bioactivity. Surface plays a crucial role in biological interaction as it is the only part of contacting with the bio-environment and the surface region of a biomaterial is always different in morphology and composition from the bulk material. The surface property of the biomaterial can be modified or altered in a favorable fashion to accommodate, facilitate or promote more biofunctionality and bioactivity by mechanical, chemical, electrochemical and thermal methods such as sand blasting, shot peening, laser peening, plasma spraying, sol gel coating etc.

a) Sand blasting and surface modification:

The purpose of sand blasting is to clean surface contaminants, to increase effective surface area by roughening the surface and to produce beneficial surface compressive residual stresses. The sand blasted surface exhibits higher surface energy which indicates higher

surface chemical and physical activities and enhances fatigue strength as well as fatigue life. In general, on the macroscopic level (> 10 μm), roughness will influence the mechanical properties of the interface, while on the microscopic level 10nm to 10μm it influences the interface biology as it is of the same order in size of cells and large biomolecules. Micro rough surface allows better cellular adhesion that alters the overall tissue response to biomaterials. It also allows the adhesion of mineral ions or atoms, biomolecules and cells which form stronger fixation of bone or connective tissue [6]. Alumina (Al_2O_3) and silica (SiO_2) particles are mostly used as blasting media. Although blasting shows some advantages, it has some adverse effect like surface contamination due to blasting media and distortion of the blasted work piece due to intensity and blasting manner [7].

b) Shot peening and Laser peening

Shot peening is a process similar to sand blasting but with more controlled peening power, intensity and direction. In shot peening surface of a part is bombarded with a spherical media called shots. Each piece of shot striking the material acts as a tiny hammer, imparting to the surface small indentions or dimples. Laser peening technology is recently developed in which there nocontact, no-media and contamination free is peening. Work piece is covered with a protective ablative layer and a thin layer of water before treatment. High intensity nanosecond pulses of laser light beam striking the ablative layer generate short lived plasma which causes a shock wave to travel into work piece. The shock wave induces compressive residual stress that penetrates beneath the surface and strengthens the work piece, [8, 9], resulting in improvement in fatigue life and retarding in stress corrosion cracking occurrence.

c) Coating

Coating layer is not only required to exhibit an expected function, but also important to adhere well to the metal substrate for transferring loads. Coating of different substances like hydroxyapatite (HAp) and bioactive glass (BG) is done on titanium for proper adhesion. HAp ($Ca_{10}(PO_4)_6(OH)_2$) is a bio ceramic which resembles the mineral constitute of human bone and teeth [10]. HAp helps the metallic implants to form a real bond with the surroundings. Hydroxyapatite coating is done for enhancement of the osteoconductivity of titanium which

is potentially beneficial to patients as it shortens the medical treatment time and increases the initial stability of the implant.

BG is a bioactive material with a high potential as implant material. Reactive plasma spraying produces economically feasible BG coating for Ti and its alloy. L.L. Hench reported that bioactive glasses (BG) in SiO_2-Na_2O-CaO-P_2O_5 system have higher bioactivity as compared to hydroxyapatite so that it can be used as an alternative to hydroxyapatite [10]. Tomsia and co-worker failed to develop bioactive coated titanium alloys as, they observed cracks in coating because of thermal expansion mismatch between the glass and the substrate, generated thermal stresses which eventually led to failure of the coating[11]. Similarly Pazo *et al* reported failure of BG coating due to crystallization of glass resulting in lack of adhesion to substrate. In case of implants for orthopaedic and dental applications, the implant requires primary stability for subsequent osseointegration, which is absurd by bioactive glass coating [12]. To overcome this, many researchers used composite of BG with HAp to improve the bioactivity along with coating adhesion.

Various techniques have been reported to apply surface modification like coating of hydroxyapatite and bioactive glass on titanium alloys, such as plasma spray, magnetron sputtering, ion beam assisted deposition, chemical vapour deposition, electrophoretic deposition, electrochemical deposition, sol–gel coating, biomimetic coating and pulsed laser deposition (PLD) Laser Engineered Net Shaping (LENS) etc [13],. Typical advantages and shortcomings can be found for all the methods. [14].

Plasma spray is widely used as it produce protecting layer against wear corrosion, high temperature corrosion, high temperature load and chemical attack [15]. However in certain cases it shows poor coating –substrate adhesion and affect the long term stability of the implants. Grit blasting surface treatment prior to coating can reduce this risk during plasma spraying. The plasma spray set up consists of microwave plasma torch with two resonators. The coaxial resonator, consisting of a metallic wall and the ignition tip, yields to high electric field strength, which is necessary to ignite the plasma. The resonance frequency can be adjusted to the microwave frequency by moving the ignition tip. The cylindrical resonator is directly mounted to the waveguide and creates a homogeneous electric field along the plasma axis. In the middle of this

resonator a quartz tube is placed, which confines the plasma. Rotating gas flow is formed by arranging the gas inlets at the bottom of the coaxial resonator tangential. This gas flow stabilizes the plasma and protects the quartz tube from the hot plasma. The microwave waveguide system consists of an isolator, which prevents the magnetron from reflected microwave power, and an impedance matching element called 3-stub-tuner. In order to handle operation gases like argon and hydrogen, the plasma torch was mounted to a vacuum vessel. For the deposition of the coatings the pressure should be lower than 1 mbar to avoid dust creation in the reaction zone in front of the substrate. The plasma torch can work at low pressure, but the energy transfer between plasma and powder particles is too low to heat up the particles, because collisions take place rarely.

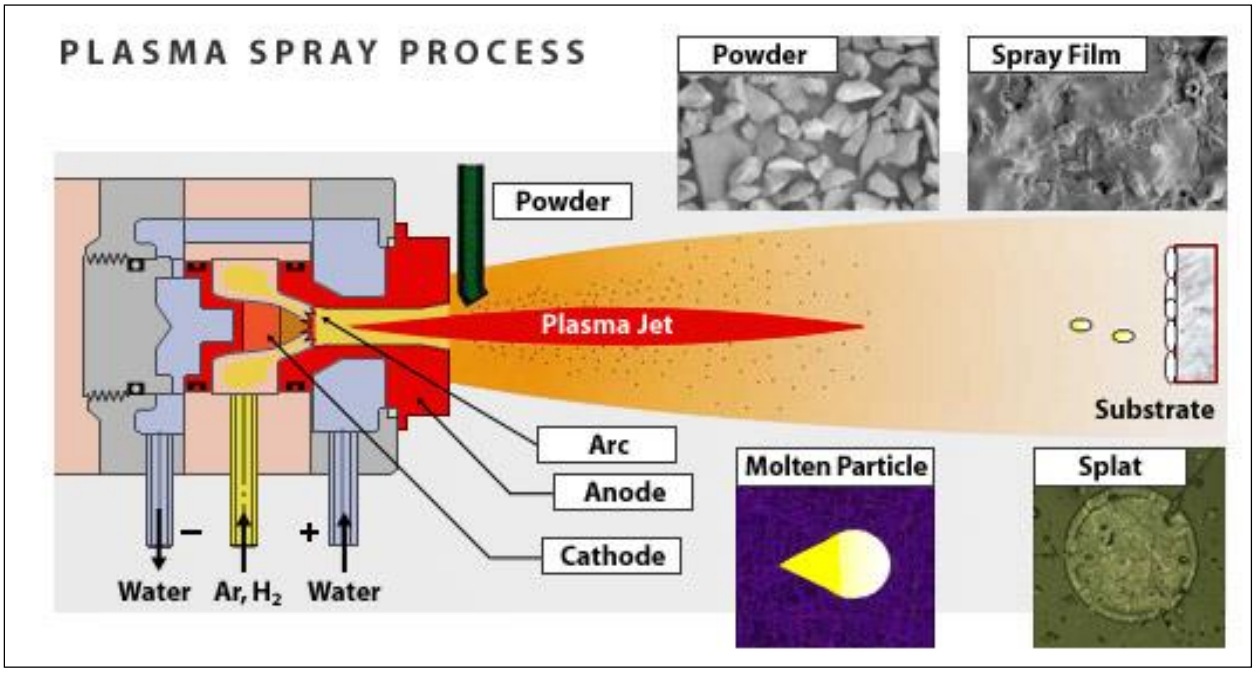

Fig 1.1: Plasma Spray Process [16]

In the present study we have used hydroxyapatite and a suitable bioactive glass containing B_2O_3 and TiO_2 for coating on commercially pure titanium using plasma spray method and its bioactivity was studied using simulated body fluid.

Chapter 2
Literature Review

Chapter 2: Literature review

2.1 Titanium and its alloys as biomaterial

Titanium and its alloys are widely used in dental and orthopedic implants. Currently pure titanium and α+β type Ti-6Al-4V ELI (Extra Low level of interstitial content) alloys are widely used as structural and/or functional biomaterials for the replacement of hard tissues in devices such as artificial total hip or knee replacement and dental implants, since they exhibits excellent specific strengths and corrosion resistance, and the best biocompatibility characteristic among metallic biomaterials. They are used more than any other titanium biomaterials, however Niiomi et al reported the use of other titanium alloys for biomedical applications [17, 18]. Among the several dozen commercially available alloys; commercially pure titanium (CpTi), Ti-6Al-4V, Ti-6Al-7Nb, Ti-3Al-2.5V, Ti-5Al-3Mo-4Zr, Ti-5Al-2.5Fe, Ti-Ni, Ti-Cu, Ti-Mo, Ti-5Al-2Mo-2Fe (SP700), are utilized experimentally and clinically tried in both the medical and dental fields. Under the category of "unalloyed grades" of ASTM specification, there are five materials classified in group of CpTi; which include ASTM grade 1 (99.5% Ti), grade 2 (99.3% Ti), grade 3 (99.2% Ti), Grade 4 (99.0% Ti) and grade 7 (99.4% Ti). Although each material contains slightly different level of N, Fe, and O, C is specified <0.10 wt. % and H is also specified <0.015 wt. %. ASTM CpTi grade 1-4 (unalloyed titanium) allow a hydrogen content up to 0.015 wt. % i.e. 15ppm. Paton et al reported that if CpTi contain more than 250 ppm of hydrogen, the material would be susceptible to stress corrosion cracking and hydrogen embrittlement [19].

Artificial materials implanted into bone defects are generally encapsulated by a fibrous tissue, leading to their isolation from the surrounding bone. However, in 1972, Hench et al. showed that bioglass whch was composed of $Na_2O–CaO–SiO_2–P_2O_5$ system, spontaneously bond to living bone. This bioglass has advantage of bonding without the formation of surrounding fibrous tissue [20]. Several types of ceramic are used clinically as important bone substitutes, such as hydroxyapatite, β-tricalcium phosphate and glass-ceramic A–W containing crystalline apatite and wollastonite. However, these ceramics are not compatible mechanically to the surrounding bone. To use this ceramic as biomaterials development of this bone-bonding materials with different mechanical properties is desired.

2.2 Hydroxyapatite

FERRAZ et al [21] reported that hydroxyapatite is the main component of mineral bone and this living bone constantly undergoes a coupled resorptive-formative process known as bone remodeling. Compared to conventional ceramic formulations HAp has more similarity with the hard tissues of the body. Due to this property HAp possesses exceptional biocompatibility and bioactivity properties with respect to bone cells and tissues. Protein interactions in body (e.g. adsorption, configuration and bioactivity) could be control by HAp properties such as surface grain size, pore size, wettability etc. therefore, modulating this properties of HAp subsequently enhanced osteoblast adhesion and long-term functionality. Webster et al discovered that these enhanced osteoblast functions are proliferation, alkaline phosphatase synthesis and calcium containing mineral deposition [22-24].

Gabriela et al [25] presents a study on an alternative coating method based on biomimetic techniques which are designed to form a crystalline hydroxyapatite layer very similar to the process corresponding to the formation of natural bone. They investigate the HAp formation on the surface of titanium alloy treated with NaOH solution using SCS (calcification solution) and M-SCS (modified calcification solution). M-SCS was very simple and highly effective and it can be successfully applied to obtain deposition of uniform coatings of crystalline hydroxyapatite on titanium substrates [25].

2.3 Bioactive glass

BG is a bioactive material with a high potential as implant material. Reactive plasma spraying produces an economical feasible BG coating for titanium and its alloy. Saiz et al [26] evaluated the in vitro response in SBF of silicate glass coating on Ti-6Al-4V. Glasses belonging to the SiO_2-CaO-MgO-Na_2O-K_2O-P_2O_5 system were used to prepare 50-70 μm thick coatings by employing a simple enameling technique. It has been found that i) coating with silica content lower than 60 wt% are more susceptible to corrosion and precipitate carbonated HA on their surface during in vitro test; however ii) these coatings have a higher thermal expansion than the metal and are under tension, iii) after 2 months in SBF, cracks grows in the coating, reaches the glass/metal interface and initiate delamination and iv) glass with silica content higher than 60 wt% are more resistant to corrosion and have lower thermal expansion, and these coating do not crack, but such

glasses with silica do not precipitate apatite even after 2 month in SBF [26]. Lee et al [27] prepared cal-ceramic, apatite-wollastonite (CaSiO3) (1:3 by volume fraction) glass ceramic, apatite-wollastonite (1:1) glass ceramic, and bioactive CaO-SiO2-B2O3 glass ceramic coating by the dipping method. Coated and uncoated Ti-6Al-4V screws were inserted into the tibia of 18 adult mongrel male dogs for 2, 4 and 8 weeks. It was found that i) at 2, 4 and 8 weeks, the extraction torque of these ceramic-coated screws was significantly higher than the corresponding torque, and ii) strong fixation was observed even at 2 weeks in all three coating except $CaO\text{-}SiO_2\text{-}B_2O_3$ glass ceramic coating [27].

2.4 Composite coating

Chern et al [28] used HAp/BG composite coating to increase the bioactivity of HAp coated Ti6Al4V and to enhance the bonding strength between coating and substrate. They observed the bonding strength of HAp was more than BG and HAp/BG composite. They also observed Open pores with sizes up to 50 μm in both BG and HAp/BG coatings, which are probably advantageous in including mechanical interlocking with the surrounding bone structure, once implanted. These HAp/BG composites could provide a coating system with sufficient bonding strength, higher bioactivity, and a significant reduction in cost in raw materials.

Silicate-based glasses with thermal expansion coefficients that match those of Ti6Al4V were prepared by Gomez-Vega et al [29] and used to coat Ti6Al4V by a simple enameling technique. BG or HAp particles were embedded on the coatings in order to enhance their bioactivity. They observed that particles with sizes over 45μm were required for enameling, otherwise the particles became hollow due to the infiltration and crystallization of the glass surface. Concentrations above 20% of surface coverage resulted in cracked coatings due to excessive induced stress.

The influence of borosilicate glass on the densification and phase change of hydroxyapatite was studied over sintering temperatures from 500 to 1200ºC by Yifei el al [30]They found that the borosilicate glass inhibited the decomposition of HA phase and enhanced the densification of the composites. When the sintering temperature was higher than 1100ºC, the glass liquid flowed away from the sample surface and was trapped inside the sample, leading to a structure of a relatively strong and dense HA/glass core and a bioactive and porous HA surface layer.

2.5 Plasma spray

Since the mid-1980s, as surface coatings on metallic implants in dentistry and orthopedics plasma sprayed hydroxyapatite (HAp) coatings were used. Geesink et al [31], Stephenson et al [32] and Cook et al [33] separately describe the advantages of plasma sprayed HAp on metal implants. Plasma sprayed HAp gives more rapid fixation and stronger bonding between the host bone and the implant moreover it increased uniform bone ingrowth.

Different researcher carried out clinical trials on plasma sprayed HAp. Although this clinical trials found little advantages in HAp-coated implants, most clinical experience shown promising results shortly after the implantation and continued fixation for upto10years with either weight-bearing or non-weight bearing models [34-38].

Soballe et al [39] reported enhance bone growth across a 1mm gap between the bone and the implant in both stable and unstable mechanical conditions, which was capable of limiting the formation of any fibrous membrane and converting a motion-induced fibrous membrane in to a bony anchorage. Reduce incidence of osteolysis and the subsequent implant failure of plasma sprayed HAp coatings have been reported as it is having good sealing effects against the migration of polyethylene particles along the bone-implant interface [40].

The bone-bonding behavior of three kinds of bioactive ceramics coated on titanium alloy by the plasma-spray technique was investigated by Toshiaki et al [41]. Titanium alloy (Ti-6A1-4V) coated with Bioglass (45S5), apatite-wollastonite containing glass ceramic (AW), or β-tricalcium phosphate (TCP) was prepared, and rectangular specimens were implanted into the tibia1 bones of mature male rabbits, which were sacrificed 8 or 24 weeks after implantation. Load failure was noted more in case of TCP than BG and AW which suggests that it is better to use a coated metal implant for short-term implantation. However, there is a possibility of breakage of the coating layer, due to both dissolution of the bioactive ceramic and mechanical weakness at the interface between the coating layer and the metal implants.

Dyshlovenko et al [42] optimized the coating of hydroxyapatite with plasma spray method followed by laser treatment. Experiment were design to study the effects of four principal parameters, i.e. electric power, plasma forming gas composition, carrier gas flow rate and laser power density onto microstructure of hydroxyapatite coatings and powders and depth of laser

melted zone. They found that electric power supplied to torch, laser power density are the two important factors which influenced HA crystal phase and α-TCP.

2.6 Simulated body fluid (SBF)

In 1991, kokubo proposed that when implanted in the living body, formation of bond like apatite on the surface of artificial implant material is essential to bond to living bone, and formation of this in vivo apatite can be reproduced in a simulated body fluid (SBF) which has ion concentration nearly equal to those of human blood plasma [43]. By apatite formation on surface of implant material in SBF one can predict the in vivo bone bioactivity of material. Since then, various types of biomaterials in vivo bone bioactivity have been evaluated by apatite formation in SBF. However, the validity of this method has not been systematically assessed.

Hench et al. reported formation of SiO_2-rich layer and calcium phosphate film on the surface of BG when implanted in the body environment, this film allows bonding to living bone. He showed that a buffer solution consisting of Tris hydroxymethyl aminomethane and hydrochloric acid (Tris buffer solution) at pH 7.4 can reproduce calcium phosphate film in vivo [44]. On the other hand, Kitsugi et al. showed that instead of SiO_2-rich layer, calcium phosphate layer forms on glass-ceramic A–W, in the living body, which allow bonding to living bone [45]. Subsequently, in 1990 Kokubo et al. found calcium phosphate layer formed on glass-ceramic surface as crystalline apatite by using micro X-ray diffraction [46]. They also showed that in an acellular SBF with ion concentrations nearly equal to those of the human blood plasma the in vivo apatite formation on the surface of glass-ceramic A–W can be reproduced, but not in a Tris buffer solution [47,48]. Kokubo et al. [47] and Hench et al. [49] confirmed the formation of apatite on the surface of Bioglass 45S5-type glass in SBF individually. Kokubo et al proved that surface apatite formed in SBF was similar to bone mineral in its composition and structure by detailed analysis in thin film X-ray diffraction (TF-XRD), Fourier transform infrared spectroscopy, scanning electron microscopy and transmission electron microscopy [47,48]. Apatite formation on the surface is due to proliferation and differentiation of oseteoblast, thus it might bond to the surrounding bone and to the apatite which was on surface, resulting into tight chemical bond between biomaterial and the living bone. Glass-ceramic A–W (Al), shows no apatite formation on its surface, both in vivo and in SBF though it contains apatite and wollastonite. This apatite and wollastonite in A-W is present in a glassy matrix which contain Al_2O_3. Due to this apatite is not

formed on surface of A-W both in vivo and in SBF resulting in no bonding with living bone [47, 50]. This study showed that for material to bond with living bone apatite formation on its surface is essential this apatite formation can be reproduced in SBF.

Research of Gamble et al suggested that original SBF used by Kokubo et al and Hench et al lacks the SO_4^{2-} ions contained in human blood plasma [51]. In 1991, Kokubo corrected this mistake and use SBF with SO_4^{2-} ion concentration similar to human blood plasma which was termed as corrected SBF [c-SBF] [43, 52]. In 1995 Cho et al [53] reported detailed procedure to prepare SBF as it is a highly saturated solution and difficult to prepare SBF without precipitation. As compared to human blood plasma c-SBF is richer in Cl^- ion and poorer in HCO3- ion. In 2003, Oyane et al [54] prepared a revised SBF (r-SBF) in which the concentrations of Cl^- and HCO^{3-} ions were, decreased and increased respectively, to the levels of human blood plasma. This r-SBF was supersaturated with respect to apatite and calcite formation due calcium carbonate as it has a strong tendency to precipitate from this SBF [55]. In 2004, Takadama et al. proposed a newly improved SBF (n-SBF) with decreased Cl^- ion concentration to the level of human blood plasma, and $HCO3^-$ ion concentration equal to that of the corrected SBF (c-SBF) [56]. For its stability and the reproducibility of apatite formation this n-SBF was compared with c-SBF. From result it was confirmed that the c-SBF have same stability and reproducibility as n-SBF [56].

Chapter 3
Materials and Methods

Chapter 3 Materials and Methods

3.1 Specimen preparation

Commercially pure titanium (Cp-Ti) was used as the substrate material. Sand blasting was carried out on the substrate for 5 min for inducing surface roughness and the substrate was cut into small specimens of 1x1x0.2cm using a Metco Baincut Med abrasive cutter. The specimens were then washed with distilled water and with acetone in a sonicator for 15 min. Roughness measurement was done on the sand blasted specimens using a non-contact profilometer (Brucker-Contour GT).

3.2 Preparation of bioactive glass powder

Bioactive glass powder was prepared as per the protocol reported by Ghosh S.K et al [57]. The chemical composition of the bioactive glass is shown in Table 1. The bioactive glass was ball milled for 8h at 200 rpm to get the desired size for the coating. The ball milled powder was dried at 50°C for 24h. and characterized for particle size and phase analysis.

Table 3.1: Oxide composition of bioactive glass, BG

Components	SiO_2	Na_2O	CaO	P_2O_5	TiO_2	B_2O_3
BAG **(mol%)**	57–60%	9–11%	21–24%	2–3%	0.5–1.5%	2–3%

3.3 Composite preparation

For coating on titanium substrate, hydroxyapatite (HAp) and bioactive glass (BG) composition was varied. Three different composite was prepared, coded and tabulated as shown in Table 2, For preparation of 5% and 10% composite of hydroxyapatite and bioactive glass, the samples were dry mixed and ball milled for 1 h to form homogenous composite powder which was then dried at 50°C. For proper mixing, composites were subjected to heat treatment in a furnace at 780°C for an hour so that the bioactive glass would adhere to the hydroxyapatite powder easily. After heat treatment, the composite was collected through a sieve of 230(BS) mess size for obtaining desired particle size for coating.

Table 3.2: Sample code with their composition

Sr. No	Sample code	Composite composition
1	H-35	HAp - 35A Current
2	H-5BG-35	95% HAp + 5% BG – 35A Current
3	H-10BG-35	90% HAp + 10% BG – 35A Current
4	H-40	HAp - 40A Current
5	H-5BG-40	95% HAp + 5% BG – 40A Current
6	H-10BG-40	90% HAp + 10% BG – 40A Current

3.4 Coating of Titanium

Coating of HAp and BG composite was done by plasma spray technique (Miller Maxstar 200SD 2.5kW) having an axial powder feeding system. Argon gas was used for plasma generation. Coating was performed on sand blasted surface of titanium metal. For coating, 2kW power was used by varying the current (35A and 40 A) and maintaining a feed rate of 7.3 g/min. Coating was done varying the current parameter for HAp, HAp + 5% BG and HAp + 10% BG as shown in table 2.

3.5Characterization

3.5.1 X-Ray powder diffraction

Coated sample was characterized using X-ray powder diffraction crystallography (PANalytical - Xpert 3040Y00). The scan range (2θ) between 20-80° with a step size of 0.05step per second. Similarly XRD was also performed after in-vitro bioactivity studies in simulated body fluid (SBF) to confirm apatite formation.

3.5.2 Optical Microscope

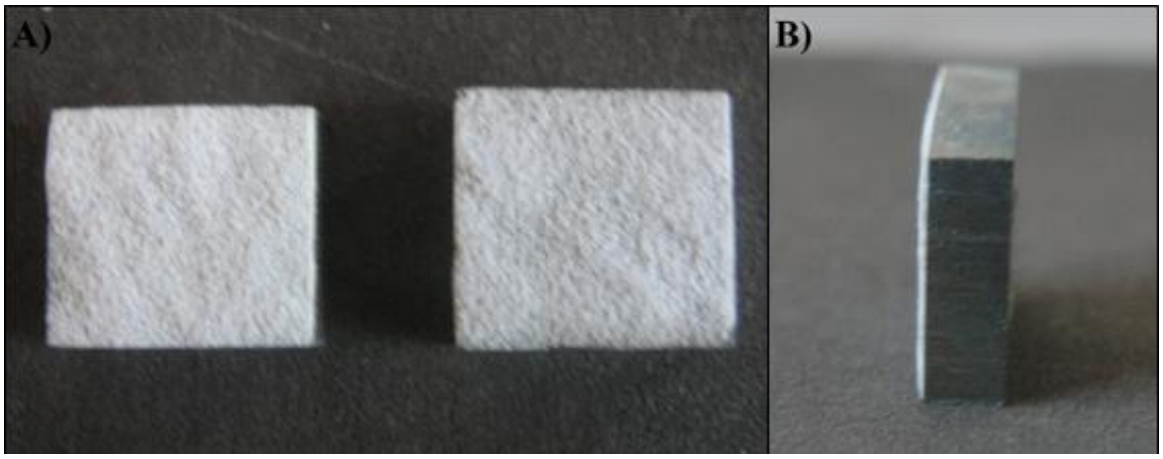

Fig 3.1: Coated Specimens A) Top View B) Side view

Coated specimens were sectioned using a slow bain cutter, mounted and observed for microstructure analysis using an optical microscope (Leitz, Germany). Cross section of specimen was analysed for coating thickness on titanium substrate.

3.5.3 Scanning electron microscopy

The plasma spray coated specimens were observed in scanning electron microscopy (SEM, (Jeol JSM – 6480LV) to study the morphology of the coated substance. SEM was also carried out after in-vitro bioactivity studies to study the morphology and growth of apatite formation when immersed in SBF

3.6 Bioactivity studies

3.6.1 In-vitro study

The bone bonding ability of the plasma sprayed specimens were evaluated by examining the ability to form apatite on its surface when immersed in simulated body fluid (SBF). Ion concentration of SBF is almost same as human blood plasma. For current study we have used corrected SBF whose ion concentrations are given in Table3 [58].

Table 3.3: Ion Concentration of blood plasma and SBF

Ion	Na^+	K^+	Mg^+	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}	pH
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5	7.2-7.4
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5	7.4

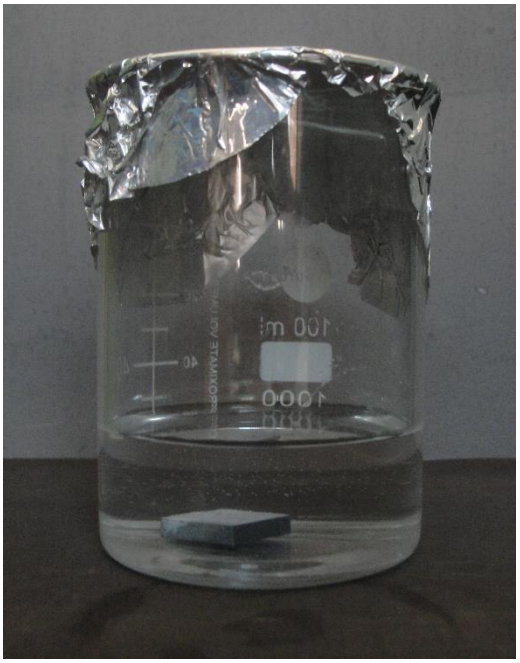

Fig 3.2: Sample immersed in SBF

As SBF is supersaturated with respect to apatite, an inappropriate preparation method can lead to the precipitation of apatite in the solution. For preparation of SBF ion concentration was dissolved in limited amount of distilled water (D/W) with stirring bar into plastic beaker at 36.5 ± 1.5° C. Reagent used for SBF was dissolved in order of NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, 1.0 M HCl, $CaCl_2$, Na_2SO_4. Then tris buffer was dissolved with small amount of 1.0 M HCl for adjusting the pH. After SBF was prepared, the coated specimens were immersed into SBF solution for 2 weeks. Apatite formation on the coated specimens was characterized on 7^{th} and 14^{th} day using XRD and SEM techniques.

Chapter 4
Results and Discussions

Chapter 4: Results and Discussions

4.1 Roughness measurement

Roughness measurement was measured on the sand blasted titanium specimen. As bioactivity of titanium surface is not high enough to induce the direct growth of bone tissue and good bone fixation, so surface treatment was required to increase its bioactivity. Surface coating like HAp/BG coating was done to increase the bioactivity of titanium substrate. Stability of this surface coating depends on proper anchoring of ceramic materials on metal substrate. For this purpose sand blasting was done to increase the surface roughness of titanium substrate. Surface roughness was measured at eight different points using non-contact method. The typical surface roughness measurement was shown in figure 4.1. The average surface roughness (Ra) of titanium substrate after sand blasting was found to be 3.66 ± 0.3 µm. This average roughness value is the mean of eight observations taken at different locations.

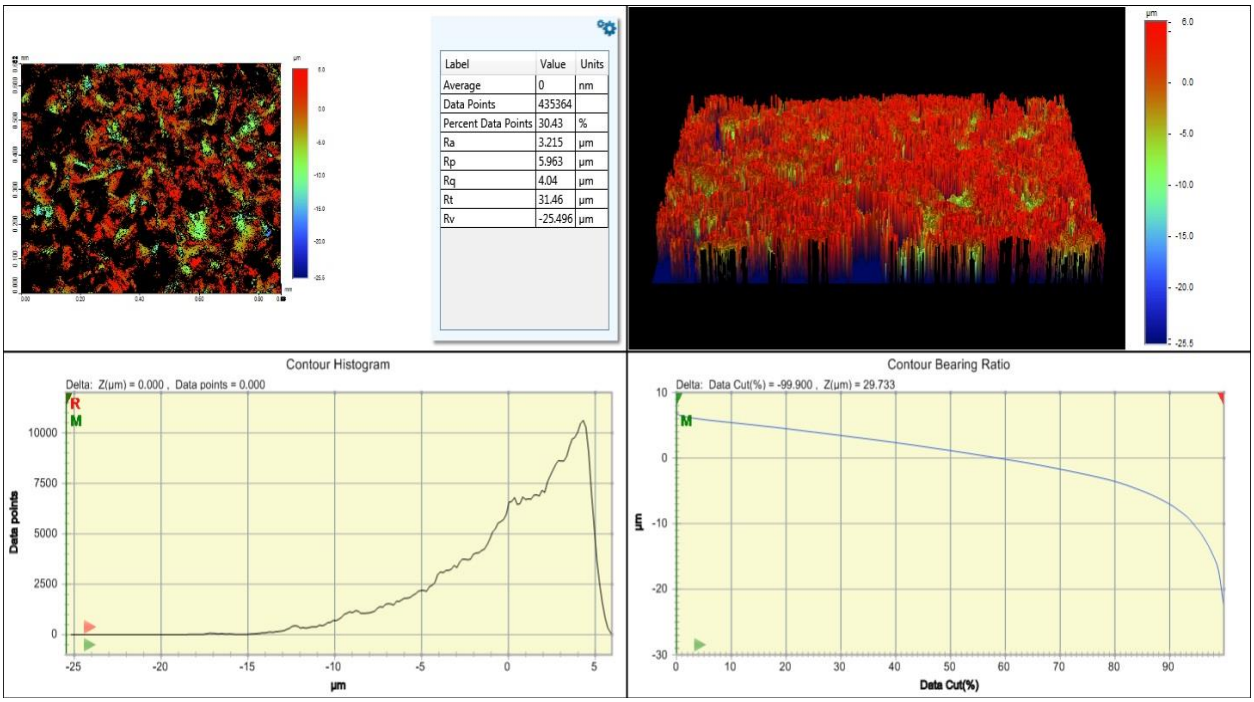

Fig 4.1: Typical surface roughness measurement on sand blasted titanium surface

4.2 Particle size analysis

Particle size of ceramic material plays very important role in coating. Smaller particles are not able to flow easily and smoothly through feeder causing the uneven coating on the surface. Similarly larger particles may not adhere on to the surface properly causing instability. In plasma spray technique arc of the plasma spray linked to the particle size and carrier gas flow. In plasma spray process as particle size decrease, the carrier gas velocity has to be increased drastically. Carrier gas velocity is proportional to negative third power of the particle diameter. Plasma spray jet is drastically disturb by the carrier gas flow rate if particle size is below 5-10µm [59]. To overcome this optimum particle size of 63µm for proper coating and stability was used. Ball milling was performed on HAp and BG powder and it was passed through proper mesh to obtain uniform particle size for coating.

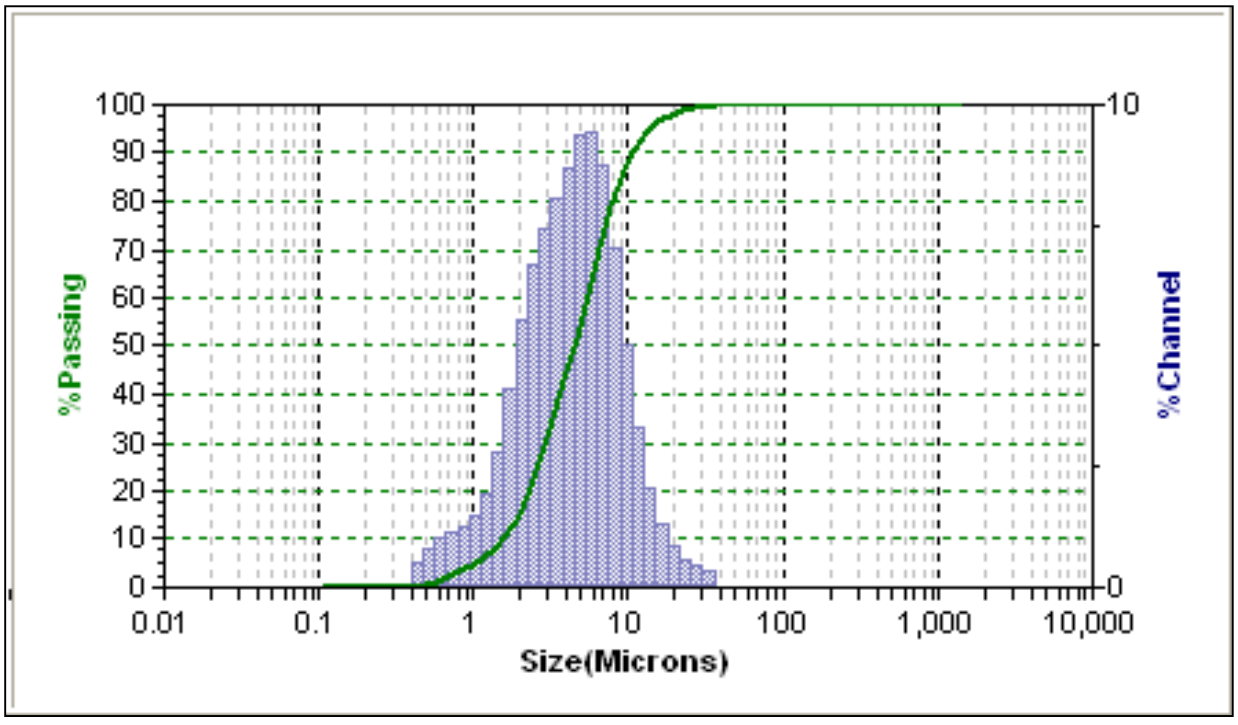

Fig 4.2: Particle size distribution of BG after ball milling

Figure 4.2 shows typical particle size analysis which was done on BG powder before coating. Figure shows the relative graph of particle size analysis done by microtac software. For this graph midpoint of channel sizes are used. Line graph was automatically developed by connecting midpoint. This graphical data provide an opportunity to view the distribution at a glance. Figure 4.2 shows that particle size ranges from 0.6µm to 80µm. Average particle size or central tendency was given by MV value which gives mean diameter in microns of the volume distribution also represents the center of gravity of distribution. MA represent mean diameter in microns of the area distribution which was calculated from the volume distribution. This area mean represents information on the distribution of surface area of the particles of the distribution. Average particle size (MV) and average area (MA) of BG was found to be 5.59µm and 3.04µm.

4.3 Characterization of coated sample

4.3.1 Xray Powder Diffraction

The XRD analysis for BG powder (figure 4.3) and HAp powder (figure 4.4) was done to check the purity of the raw material. Figure 4.3 shows amorphous nature of bioactive glass. Broad peak was observed around 20-35^0 as reported by Chidambaram et al [60]. Figure 4.4 shows XRD analysis of HAp powder. Peaks of hydroxyapatite were compared with JCPDS 74-0566 and found to be match. This indicates that standard material HAp is pure and does not contain any contamination. Figure 4.5 shows XRD pattern of various surface coated specimens (6 specimen as coded in table 3.2). H-35 and H-40 specimen showed peaks of HAp while composite powder showed peaks of HAp along with SiO_2 and sodium calcium silicate which was compared with JCPDS 80-1296 and JCPDS 83-2300. The identification of two distinct phases in the composite coating of HAp/BG, with composition similar to BG powder used for coating indicate that melted particle deposited on titanium substrate as separate phase. SiO_2 and sodium calcium silicate peaks in composite coating also indicates that crystallization occur in the BG phase Peaks of XRD shows crystallinity of the coating containg HAp is imperfect and decrease as the amount of HAp in composite powder decrease [61].

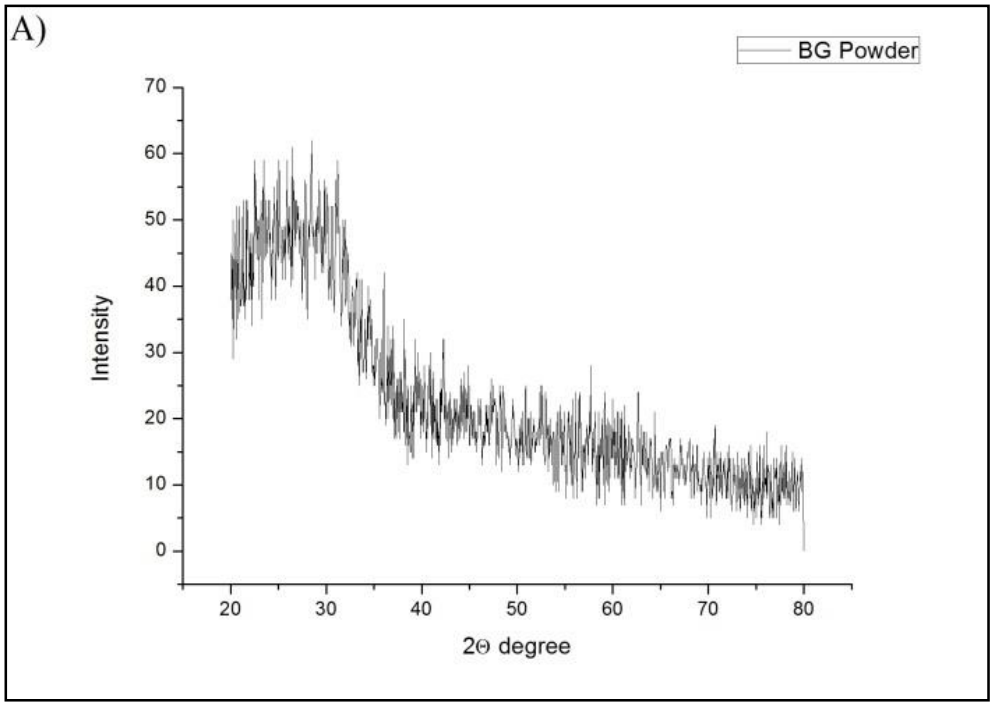

Fig 4.3: XRD analysis of BG powder

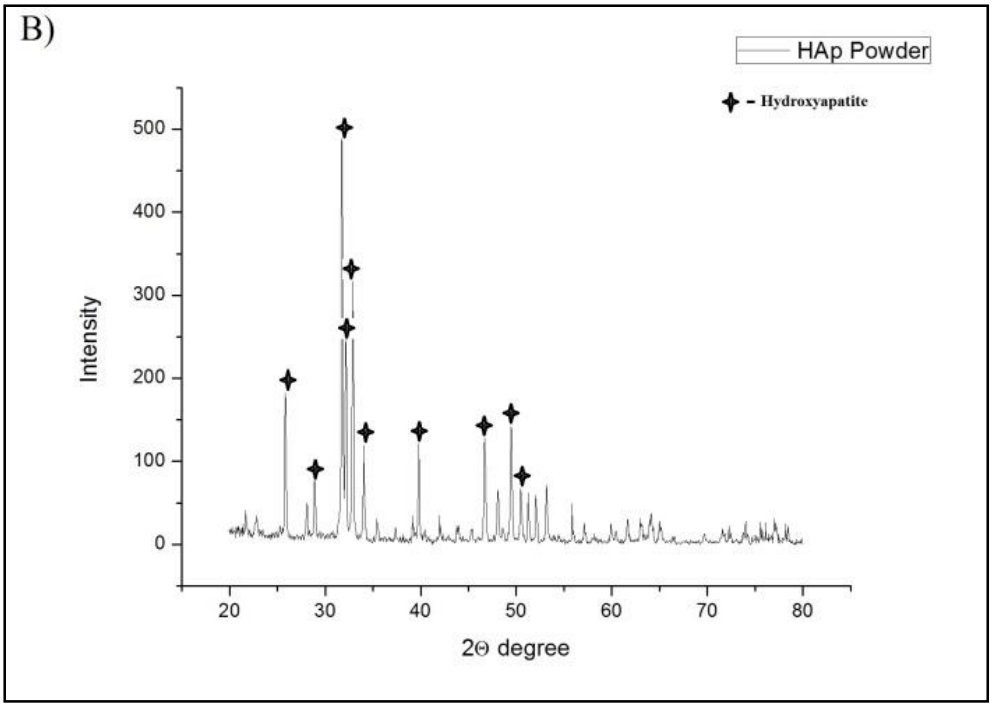

Fig 4.4: XRD analysis of HAp powder

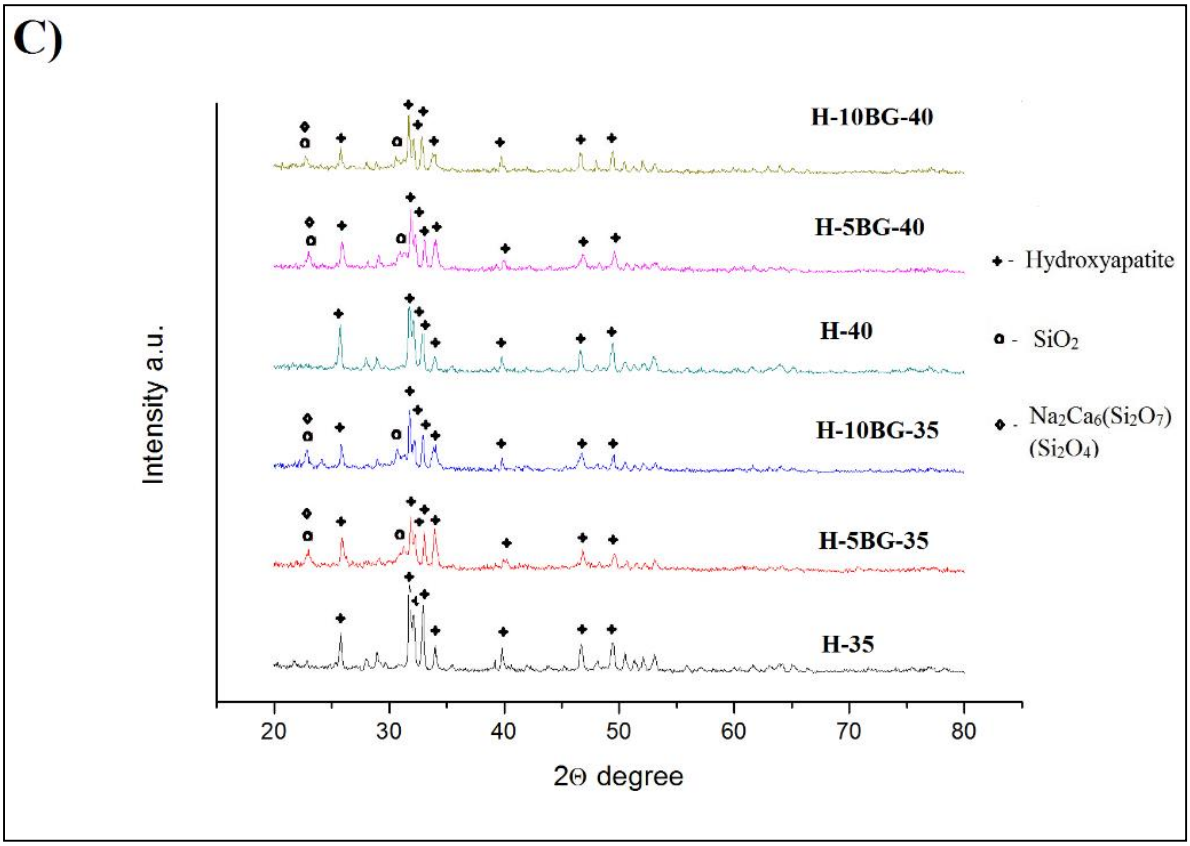

Fig 4.5: XRD analysis of processed specimen

4.3.2 Optical Microscope

Optical micrograph of plasma sprayed coated specimen is shown in figure 4.6. In figure yellow part is of titanium substrate while in black part shows the polymer in which specimen was mounted, coating was observed in between metal and polymer. As HAp and BG is of ceramic nature, with normal transmitted light it showed a translucency. Because of this translucency coating was not clearly observed without staining [62]. In figure 4.6 broad coating layer was observed in case of HAp coating while in case of composite coating, layer is thinner. Coating thickness is depend on the surface roughness and composition of powder. It shows that in case of HAp, coating was properly spread on the surface of the titanium substrate.

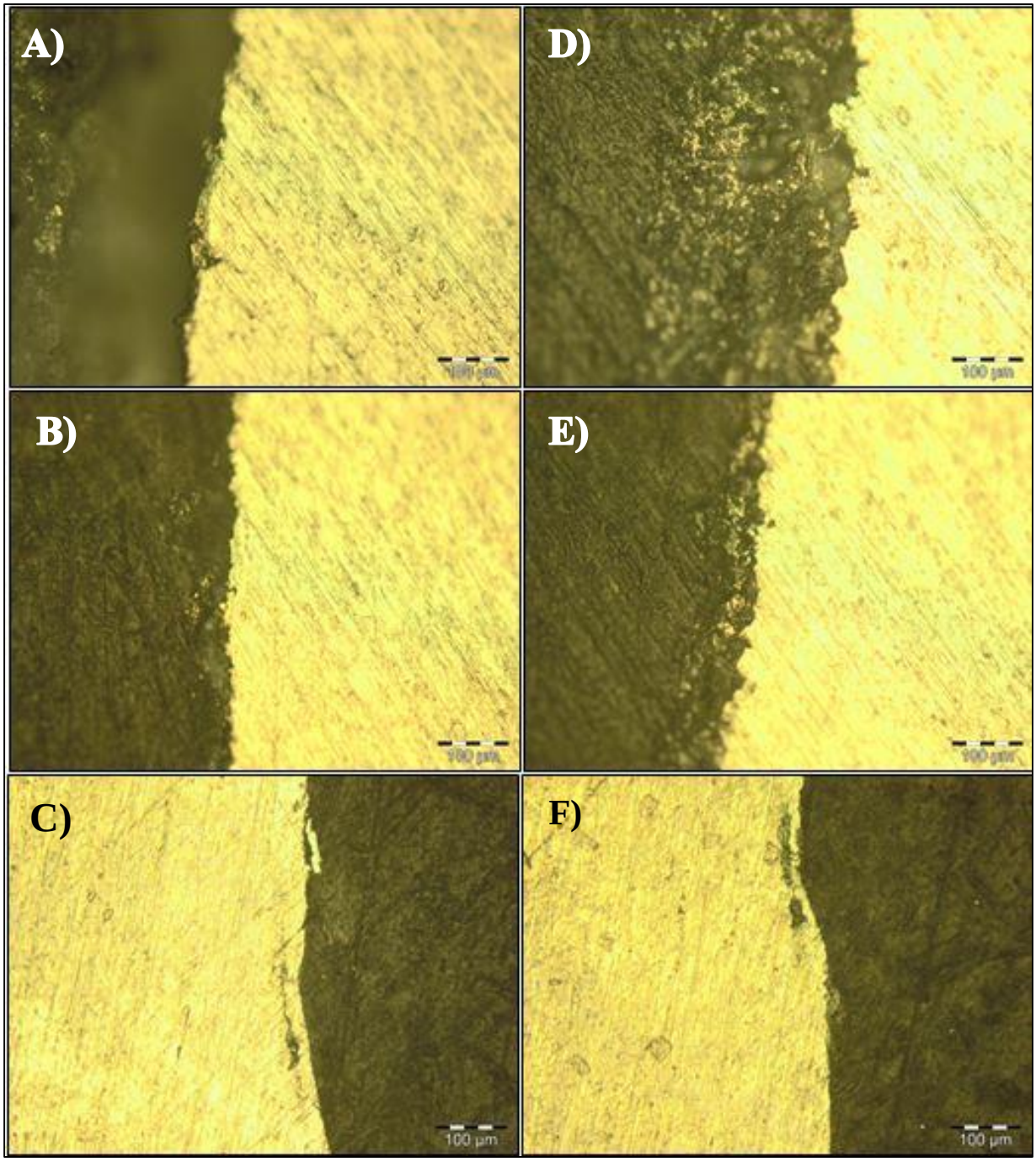

Fig 4.6: Optical micrograph of plasma sprayed coated specimen A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

4.3.3 Scanning electron microscopy

The surface morphology of the various coated specimen was obtain in SEM as shown in figure 4.7, 4.8 and 4.9. It shows rough and melt like structures on the surface of titanium substrate. Many splats can be observed on the rough surface of the coating which resulted from the impingement of the melted powder.

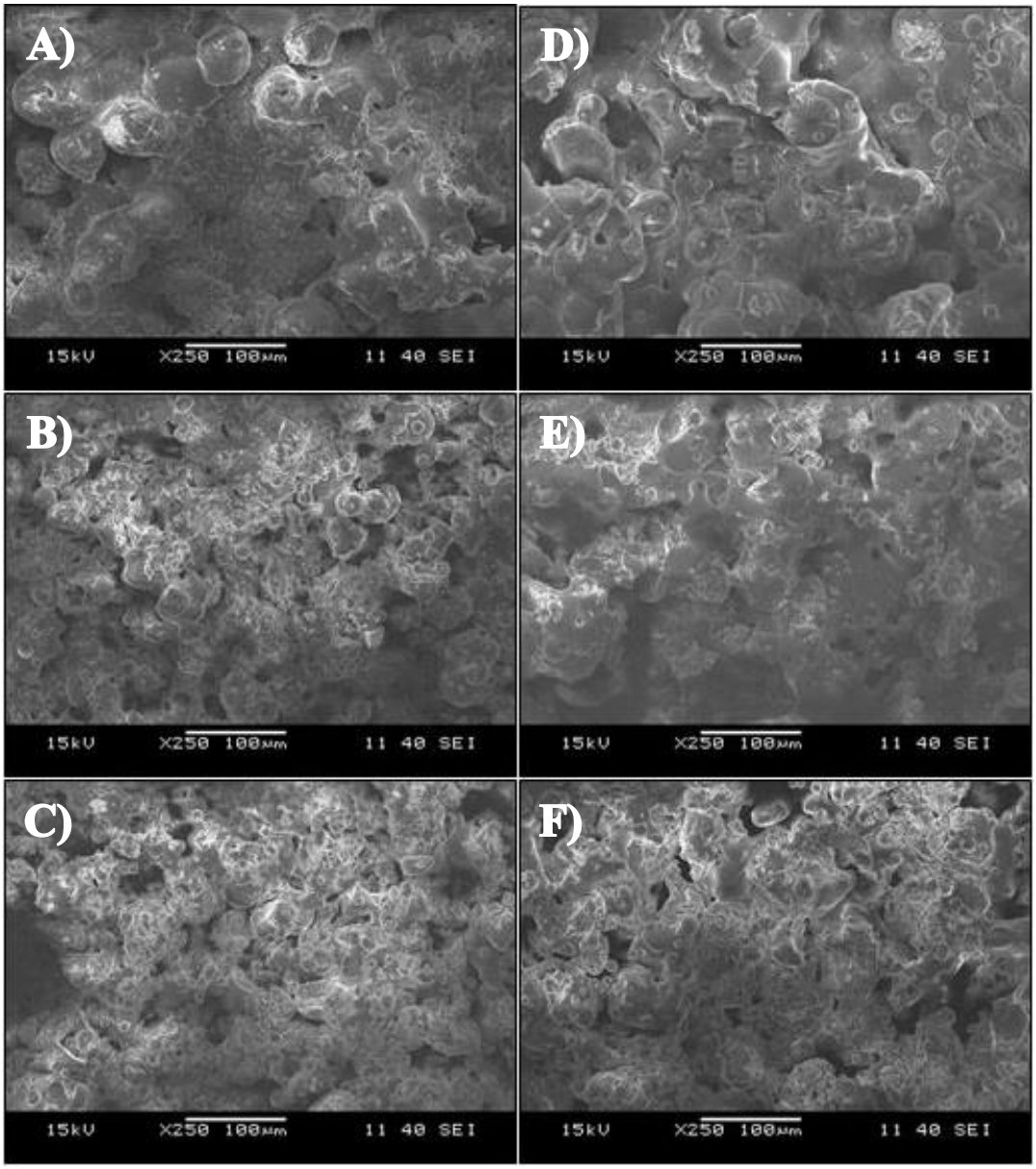

Fig 4.7: SEM micrograph of plasma sprayed titanium specimen at 250X A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

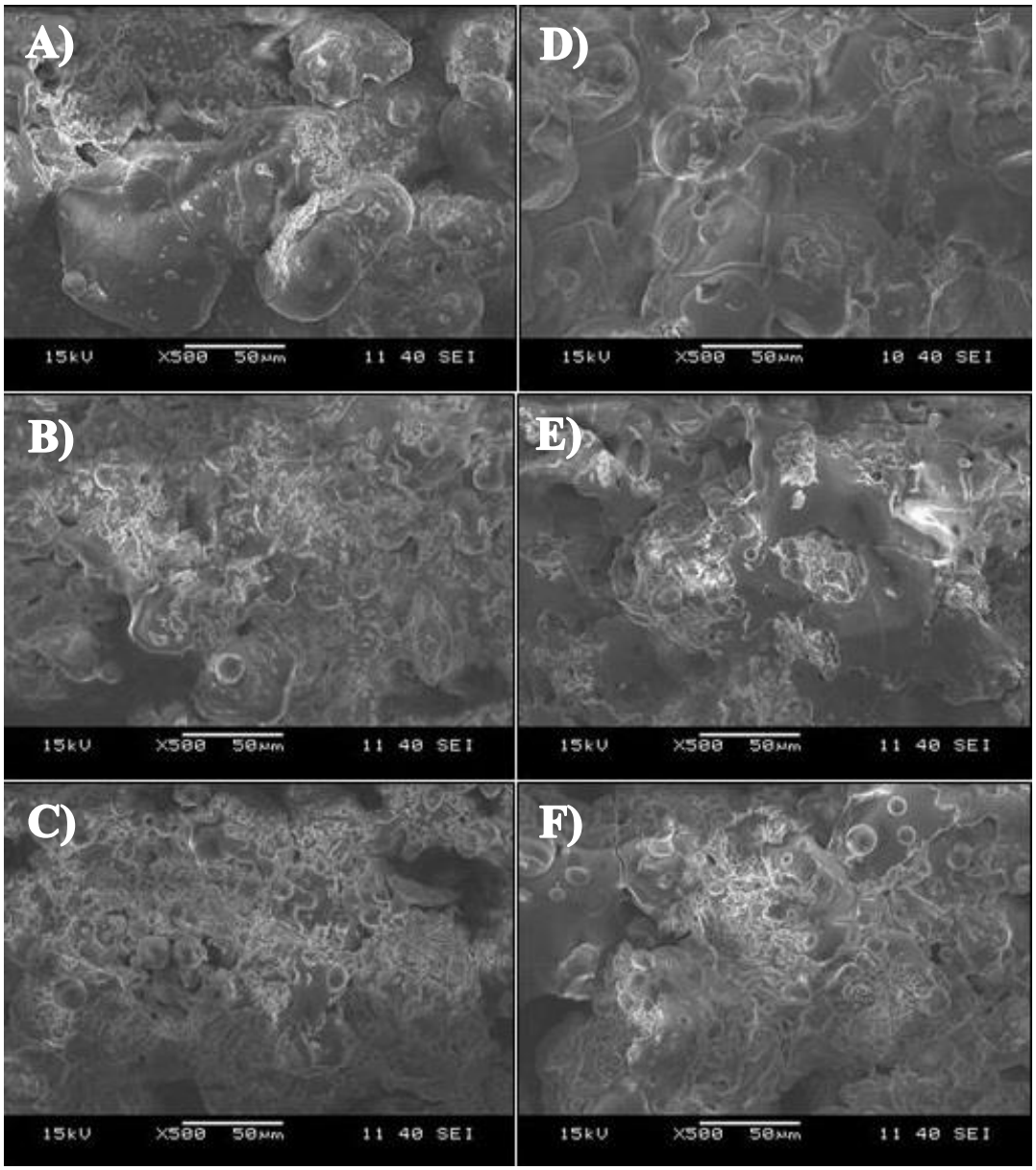

Fig 4.8: SEM micrograph of plasma sprayed titanium specimen at 500X A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

Surface of the composite coating shows more pore as compared to HAp coating. This is due to decrease of the viscosity of the BG and the escaping of the gas at higher temperature. Surface area of the coating is greatly increase by this open pores. Bonding between the implant and the surrounding bone structure get enhance as more surface area provide favorable sites for bone

ingrowth. The densities of the coatings, the quality and the size of open pores all decrease with the increase of HAp content. Coating with the lower HAp content can be well spread out on and tightly bonded to the Ti substrates. But coating with more than 30 wt% BG with HAp resulted into very loose and weak coating, even when it was heat treated at 900° C [63].

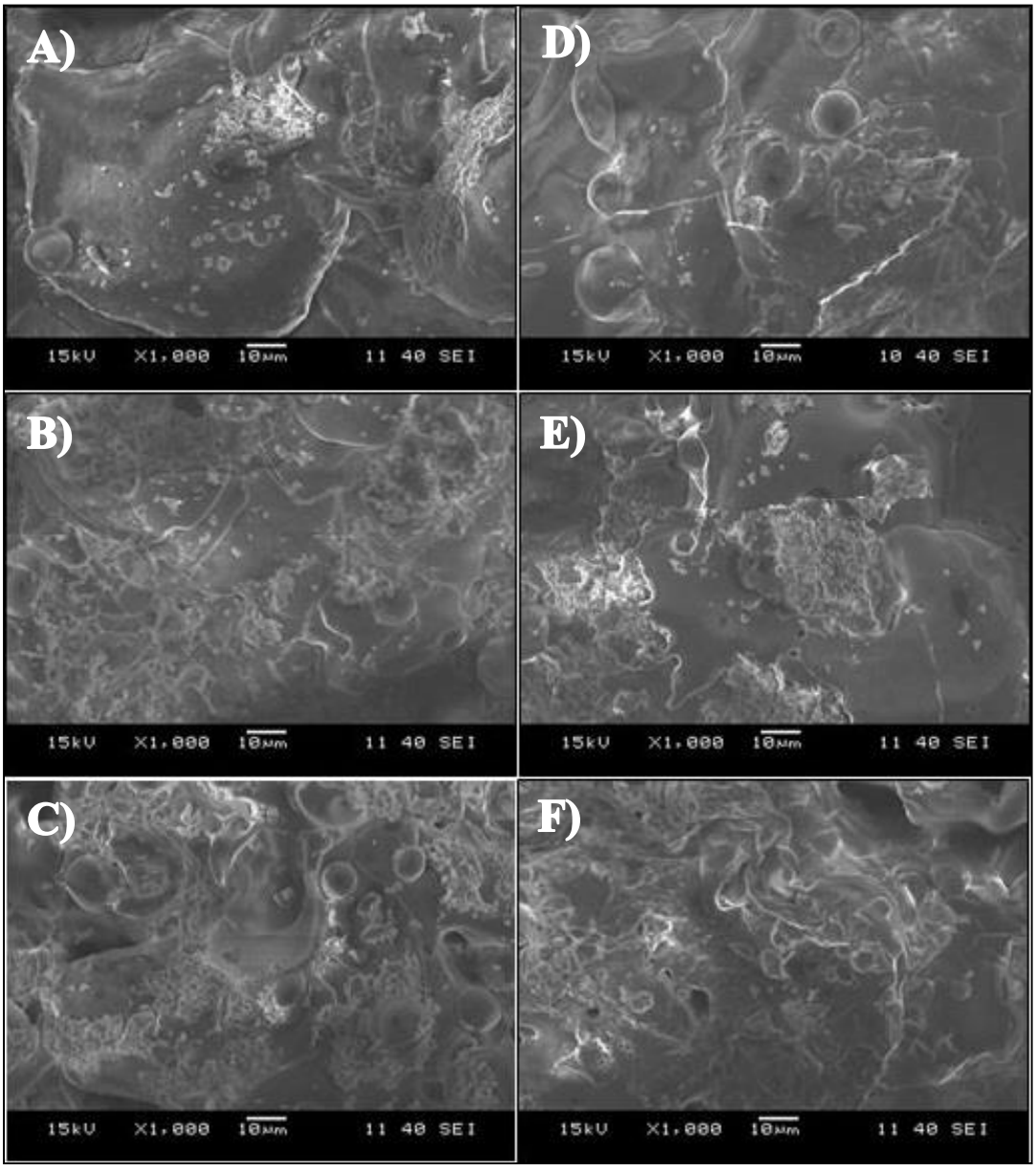

Fig 4.9: SEM micrograph of plasma sprayed titanium specimen at 250X A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

Microcracks were observed on surface of coated sample (figure 4.9) due to mismatch of thermal expansion between the coating and substrate and release of thermal stress during cooling. This microcracks were visible at higher magnification. The surface cracks is due to high coefficient of thermal expansion in HAp/BG coating when compared to titanium substrate. The coating experience tensile residual stress and the substrate experience compression residual stress [64]. To reduce the surface cracks it is important to control the spray parameter such as current, feed rate etc.

4.4 In-vitro bioactivity studies

4.4.1 Scanning electron microscopy

In-vitro bioactivity studies were performed by immersing the coated specimen in SBF for 7 and 14 days. To confirm the apatite formation on the surface of coated specimen XRD and scanning electron microscope was used. Figure 4.10-4.15 shows the SEM micrograph of specimen immersed in SBF for 7 and 14 days at different magnification. SEM micrograph shows dissolution of sample after immersing in SBF, this dissolution get increase with respect to time. Dissolution of coated sample shows surface roughness. Wang et al reported same phenomenon for plasma sprayed HAp coating after soaking in SBF for few hours **[**65**]**. Surface roughness allows bone like apatite formation to anchor as it provides nucleation site with lower interface energy. As immersion time increases, tiny granular precipitates have deposited on the specimen surface. Immersed sample shows more microcracks on the surface as compared to processed sample. Due to diffusion reaction between the coating surface and SBF this cracks are formed. Diffusion of ions from the coating surface to surrounding SBF also formed some micropores [64]. After 2 weeks of soaking the surface of the coating was covered by a newly formed layer consisting of small granular structure. The quantity and size of the precipitates are directly related to immersing time. As soaking duration increase this granular layer becomes denser and granules in the layer become larger. At higher magnification it can be observed that this layer is composed of many small crystallites. These small crystallites suggest the high nucleation rate of apatite which grows with time and later acts as a nucleation site for other apatite crystallites. It was observed that the growth of apatite was more in HAp/BG composite (5% and 10%) coating than HAp coating. Similarly specimen coated at 35A current formed more apatite formation than the specimen coated at 40A current.

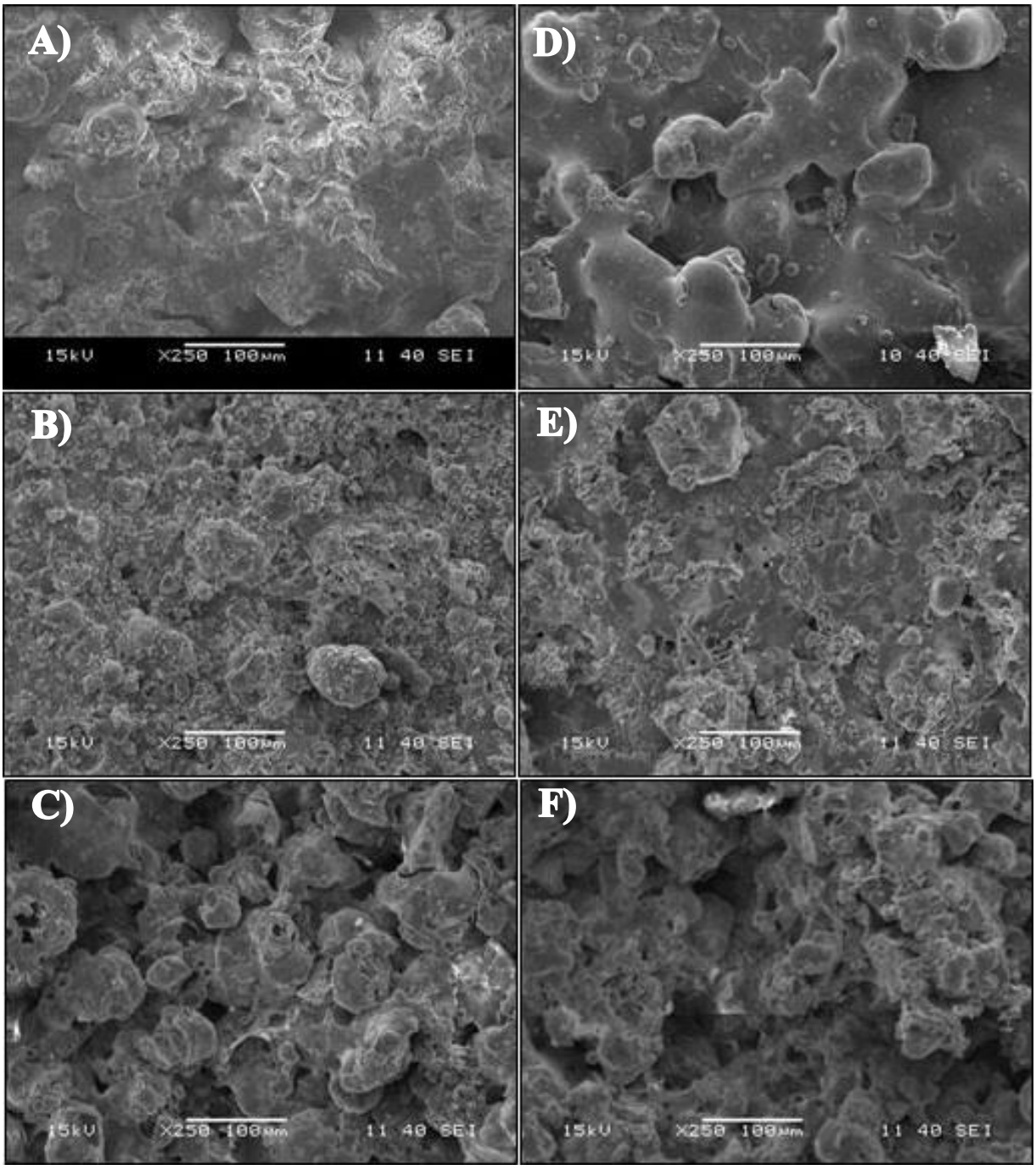

Fig 4.10: SEM micrograph at 250X of plasma sprayed titanium specimen immersed in SBF for 7 Days A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

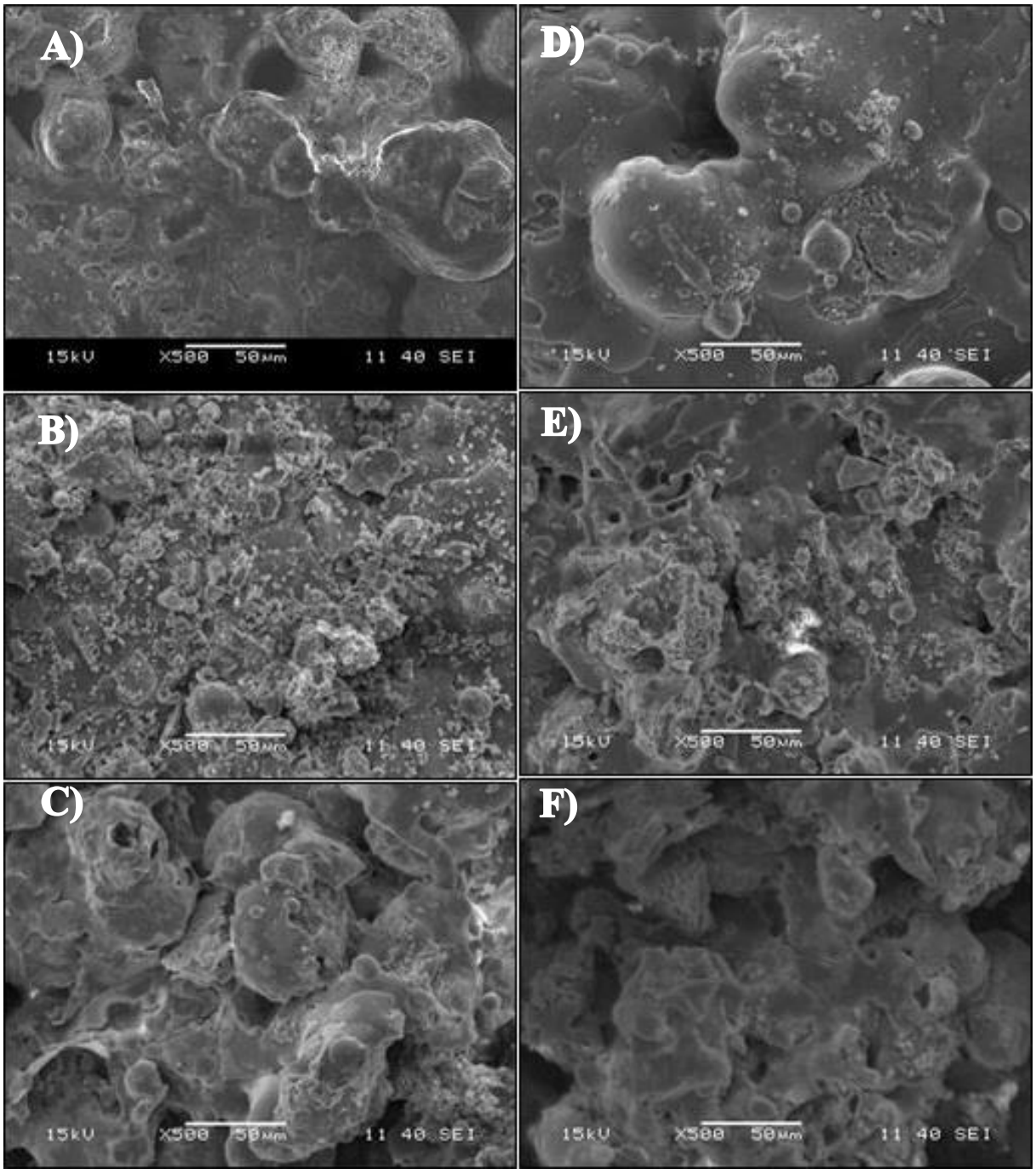

Fig 4.11: SEM micrograph at 500X of plasma sprayed titanium specimen immersed in SBF for 7 Days A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

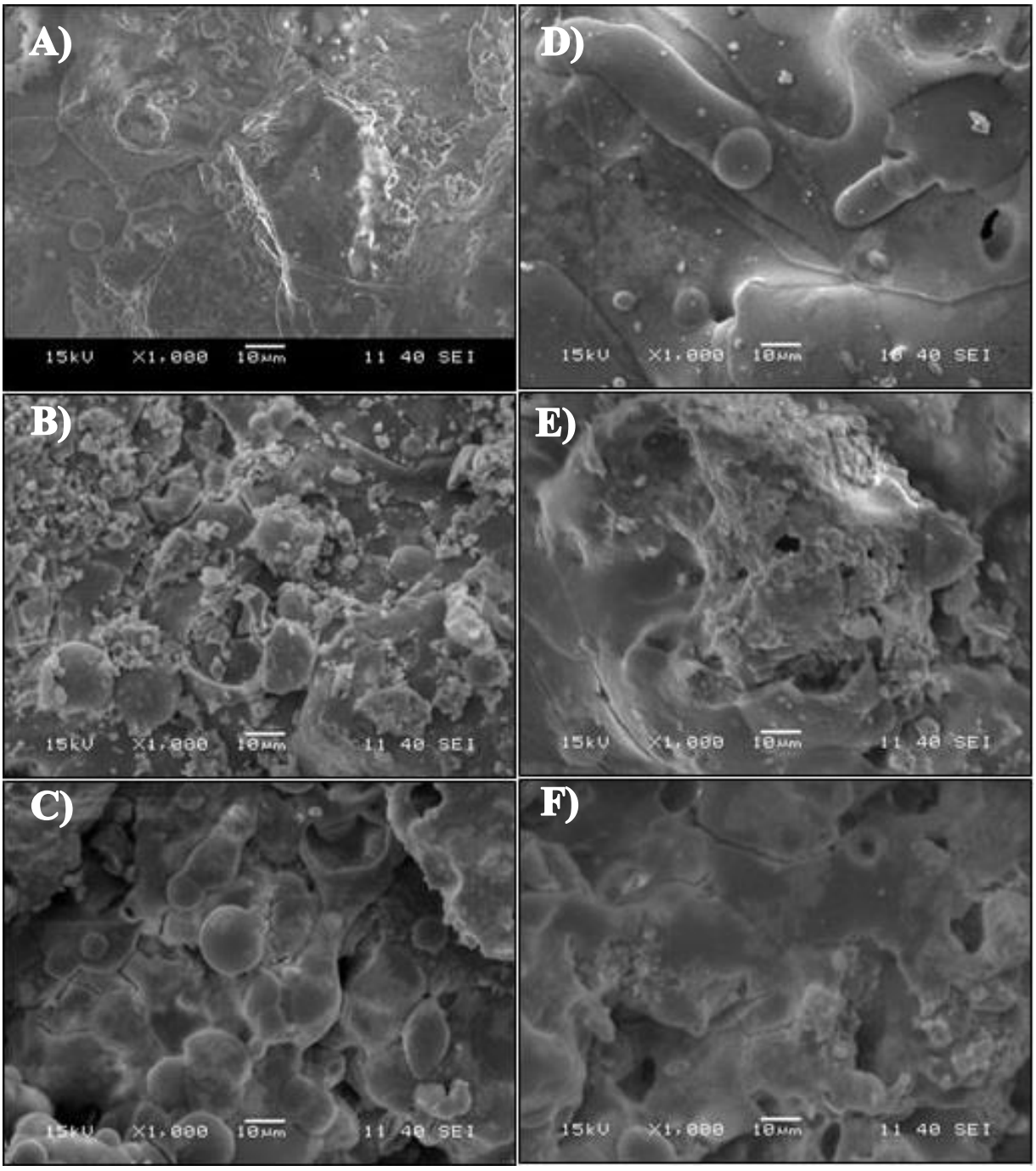

Fig 4.12: SEM micrograph at 1000X of plasma sprayed titanium specimen immersed in SBF for 7 Days A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40,E) H-5BG-40 and F) H-10BG-40

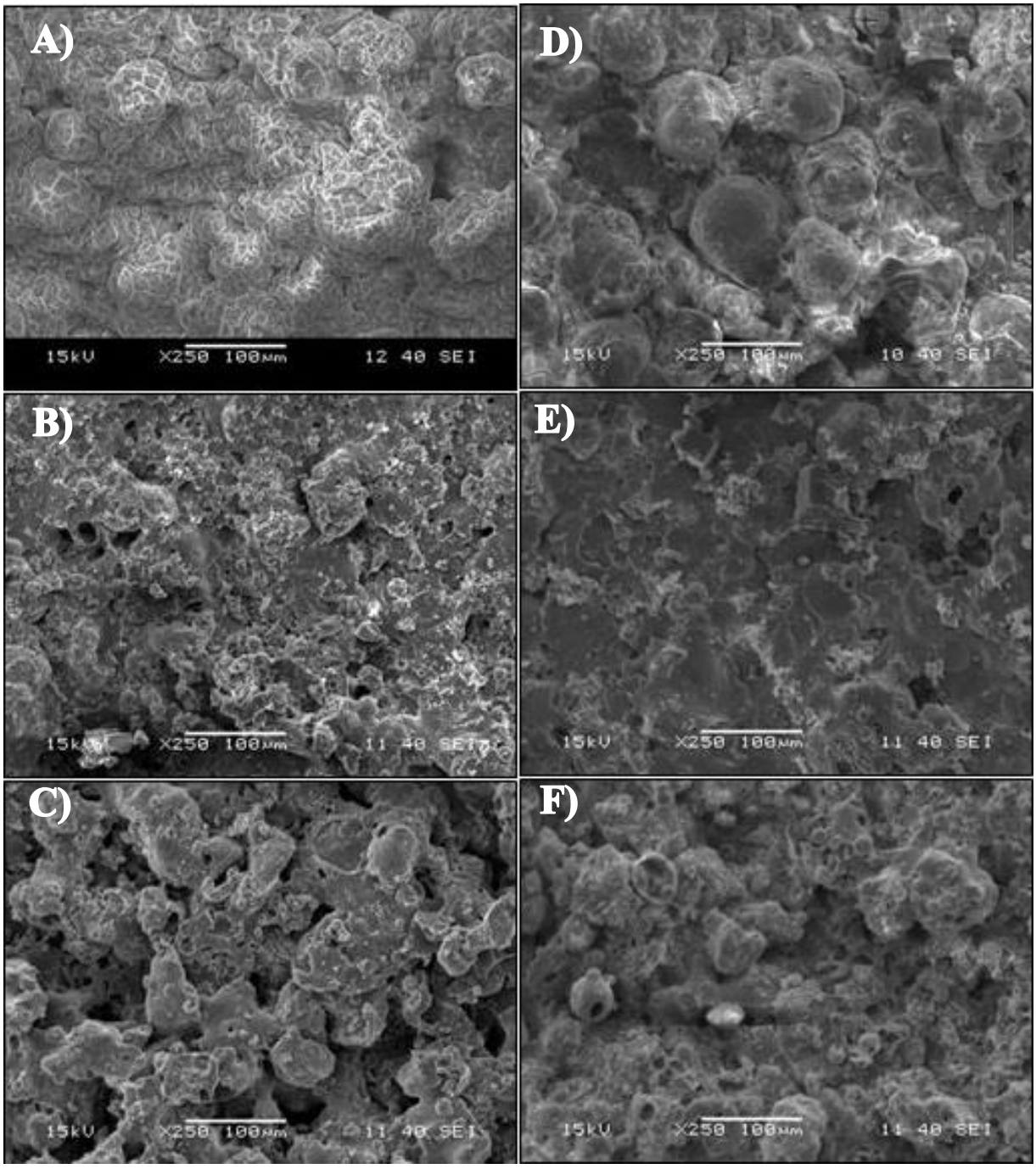

Fig 4.13: SEM micrograph at 250X of plasma sprayed titanium specimen immersed in SBF for 14 Days A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

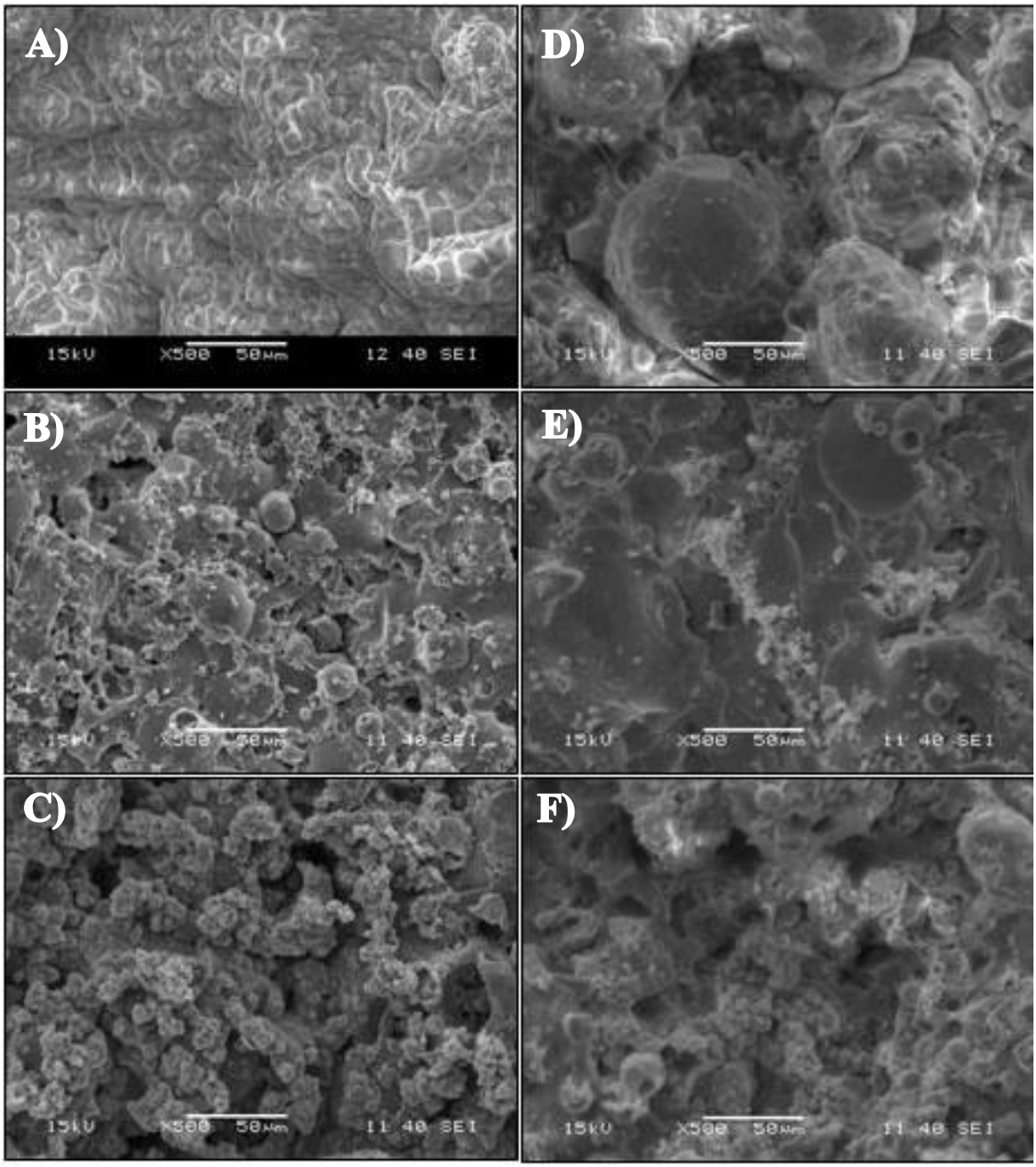

Fig 4.14: SEM micrograph at 500X of plasma sprayed titanium specimen immersed in SBF for 14 Days A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

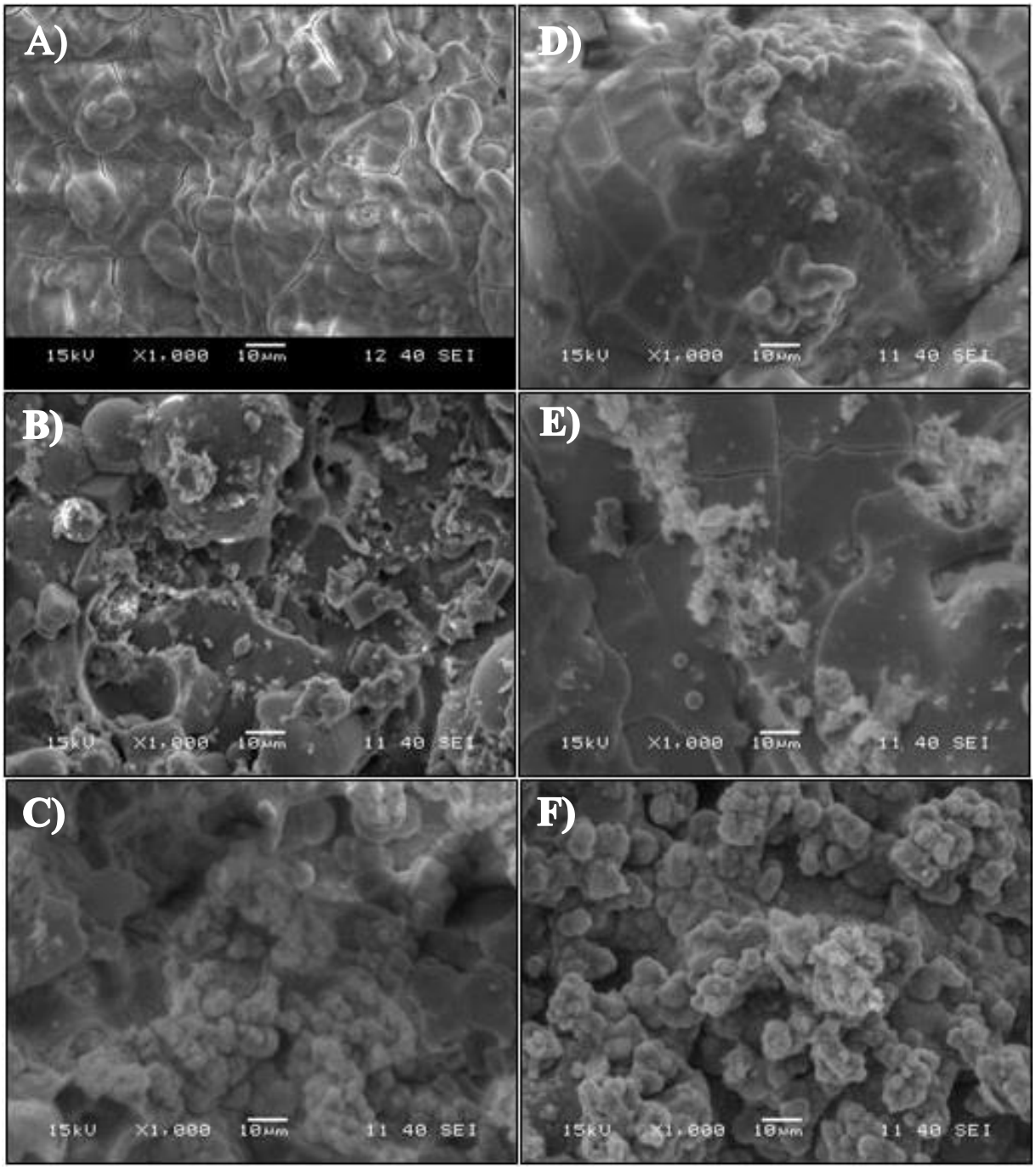

Fig 4.15: SEM micrograph at 1000X of plasma sprayed titanium specimen immersed in SBF for 14 Days A) H-35, B) H-5BG-35, C) H-10BG-35, D) H-40, E) H-5BG-40 and F) H-10BG-40

SEM micrograph shows that bone apatite were formed on the surface of HAp and HAp/BG coating after immersing in SBF. The bone-bonding interface between bioactive materials and living tissue can be formed by this apatite formation on surface which suggests increase longevity of coating during implanting in-vivo.

Summary and Conclusion

Summary and Conclusion

- Different concentrations of HAp-BG were mixed and coated on titanium substrate using plasma spray technique.
- During coating the current parameter was varied to 35A and 40A.
- SEM micrograph shows micropores on the surface of coated specimen which is due to decrease in viscosity of bioglass and the escaping of the gas at higher temperature.
- XRD analysis did not show any phase contamination after plasma spray coating.
- In-vitro bioactivity study in SBF showed dense apatite formation on all the specimens.
- In HAp/BG coating, H-10BG-35 showed more apatite formation than rest of the composition.
- Plasma spray technique can used for coating hydroxyapatite and bioglass on titanium substrate if its processing parameters are carefully chosen.

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